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The Membrane Attack Complex of Complement Causes Severe Demyelination Associated with Acute Axonal Injury

Richard J. Mead,* Sim K. Singhrao,* James W. Neal,† H. Lassmann,‡ and B. Paul Morgan2*†

Complement is implicated in pathology in the human demyelinating disease multiple sclerosis and in animal models that mimic the demyelination seen in multiple sclerosis. However, the components of the complement system responsible for demyelination in vivo remain unidentified. In this study, we show that C6-deficient (C6−) PVG/c rats, unable to form the membrane attack complex (MAC), exhibit no demyelination and significantly reduced clinical score in the Ab-mediated experimental autoimmune encephalomyelitis model when compared with matched C6-sufficient (C6+) rats. In C6+ rats, perivenous demyelination appeared, accompanied by abundant mononuclear cell infiltration and axonal injury. Neither demyelination nor axonal damage was seen in C6− rats, whereas levels of mononuclear cell infiltration were equivalent to those seen in C6+ rats. Reconstitution of C6 to C6− rats yielded pathology and clinical disease indistinguishable from that in C6+ rats. We conclude that demyelination and axonal damage occur in the presence of Ab and require activation of the entire complement cascade, including MAC deposition. In the absence of MAC deposition, complement activation leading to opsonization and generation of the anaphylatoxins C5a and C3a is insufficient to initiate demyelination. The Journal of Immunology, 2002, 168: 458–465.

Although the etiology of the human inflammatory and demyelinating disease multiple sclerosis (MS)1 is uncertain, it is widely accepted that MS is immune mediated (1). Evidence from the animal model experimental autoimmune encephalitis (EAE) implicates an autoimmune T cell response as the initiating factor. However, in many models of EAE, demyelination and tissue damage are minor (2). Other aspects of the immune system may be responsible for myelin damage, and it has been suggested that different mechanisms of pathology are operating in different subsets of MS patients, or even within the same patient (3). In addition to myelin damage, direct axonal injury occurs in MS (4), and axonal loss correlates with persistent functional deficit (5). The mechanisms leading to axonal damage are not well understood.

A wealth of evidence has implicated the complement system in demyelination in vitro, in diverse animal models of MS and in the human disease (6–13). Early in vitro models of demyelination using cerebellar cultures showed that the demyelinating component of sera from animals with EAE was heat labile, a classical characteristic of the complement system (8). In rat EAE, complement depletion ameliorates disease (9), and in a demyelinating model, Ab-mediated demyelinating EAE (ADEAE), induced by injection of Abs to myelin oligodendrocyte glycoprotein (MOG) at the onset of clinical signs in EAE (10), complement is essential for demyelination (11, 12). Studies in C3 gene-targeted (C3−/−) mice have been contradictory. One study reported a protective effect of C3 deficiency in that C3−/−, C57BL/6 mice had reduced levels of disease, inflammation, and demyelination in MOG peptide-induced EAE (13). In contrast, a second study has reported no effect of C3 deficiency on the course of MOG-induced EAE in C3−/−, BL/6 × 129/sv F1, mice (14). In the human disease, evidence for a role of complement has been provided by analyses of complement components and activation products in cerebrospinal fluid and MS brain (6, 7, 15–17). Deposition of the membrane attack complex (MAC) was particularly evident at the edges of active plaques, suggesting a close association with ongoing pathology (16). Recently, a combined study of actively demyelinating lesions from MS patients defined four different pathological patterns. Pattern II, the most common (39 of 73 cases), was typified by high levels of Ig and MAC deposition at sites of active myelin destruction (17). Autoantibodies against MOG have been found on disintegrating myelin around axons in acute MS lesions and in a marmoset model of demyelinating EAE, suggesting that some forms of MS may resemble ADEAE (18).

Together, these data firmly implicate complement in the pathology of experimental and clinical demyelination, but tell us nothing about how complement causes damage. Activation of the complement cascade leads to a variety of biological outcomes driven by generation of the proinflammatory anaphylatoxins (C3a and C5a), deposition of complement fragments on cell surfaces (opsonization), and formation of the lytic MAC (19). To further dissect the products of complement activation that play a pathological role in demyelination, we established colonies of C6-deficient (C6−) and C6-sufficient (C6+) PVG/c rats, as previously described (20). The C6− animals are unable to form the lytic MAC, but otherwise have a functional complement system, enabling the roles of MAC and early activation products to be differentiated in disease models (21). Protocols were established for induction of EAE and ADEAE in PVG/c rats, and disease profiles were compared in C6− and C6+ PVG/c rats during the course of EAE and ADEAE.
Materials and Methods

Animals

Normal (C6−) PVG/c rats were obtained from Bantin and Kingman Universal (Hull, U.K.), C6− PVG/c rats were originally obtained from Bantin and Kingman Universal (Fremont, CA). Animals were maintained according to Home Office guidelines. For the disease models, female rats at 8–10 wk of age were used.

Antibodies

Rabbit polyclonal antisera to rat C1 and C9 were prepared as described (12). Monoclonal mouse anti-rat ED1, a rat monocyte/macrophage marker (22), and W3/13, a marker of T cell and granulocytes, were from Serotec (Oxford, U.K.). Monoclonal mouse anti-amyloid precursor protein (APP) was from Chemicon (Temecula, CA); and mouse monoclonal SMI 31, antiphosphorylated neurofilament, was from Sternberger Monoclonals (Baltimore, MD). Secondary Abs for immunocytochemistry were minimal cross-reactivity donkey anti-rabbit HRP conjugate (Jackson Immunoresearch, West Grove, PA) and rat anti-mouse IgG1 heavy chain HRP (Se-rotec). Monoclonal mouse anti-MOG (Z12) was produced as described (11) and purified from tissue culture supernatant by affinity chromatography (Prosep-A; BioProcessing, Princeton, NJ). Monoclonal mouse anti-human C6 (clone WU6-4) was kindly provided by R. Würzner (Leopold Franzens University, Innsbruck, Austria) (23).

Induction of EAE and ADEAE

EAE and ADEAE were induced essentially as described (12). Briefly, C6− and C6+ PVG/c rats were immunized in each hind footpad with 50 µl of a 1:1 emulsion of 1 mg/ml guinea pig myelin basic protein (ggMBP) in PBS and free CFA (Difco, Surrey, U.K.) containing 4 mg/ml Mycobacterium tuberculosis H37 Ra (Difco). On day 13, ADEAE animals additionally received 0.8 mg Z12 anti-MOG mAb i.p. Animals were weighed daily and monitored for clinical signs of disease, scored as follows: 0, no clinical signs; 0.5, tail weakness; 1, tail atony; 1.5, tail atony and abnormal gait; 2, hind limb weakness; 2.5, complete paralysis of one hind limb; 3, complete paralysis of both hind limbs; 4, moribund. Animals that had reached a clinical score of 3 or 4 at the time of assessment were sacrificed immedi-
ately to conform with Home Office conditions.

Purification of human C6 for reconstitution studies

Fresh frozen human plasma was obtained from University Hospital of Wales blood bank, thawed at 37°C, centrifuged, and passed over a Wu6-4 monoclonal affinity column. The column was washed with 20-column volumes of PBS/0.5 M NaCl, followed by PBS until no further protein was eluted. C6 was then eluted with 50 mM diethyamine in PBS, pH 11.5, in 5-ml fractions. Fractions were monitored for C6 activity by hemolysis assay, active fractions were pooled and neutralized, and the pool concentrated using an Amicon series 8000 stirred ultrafiltration cell (Mil-

Processing of brain and spinal cord tissue

Animals were sacrificed 14–16 days postimmunization by perfusion with perfusion with cold 4% paraformaldehyde in PBS via the aorta while under terminal an-
esthesia. Brains and spinal cords were removed, postfixd in 4% parafor-
maldehyde in PBS at 4°C overnight, washed in PBS, and embedded in parain or araldite resin using standard protocols. Paraffin sections were cut as described (24) and stained with H&E to assess inflammation, luxol fast blue/cresyl violet (LFB/CV) for demyelination, and Bielschowsky’s silver method for axonal loss. Semithin resin sections were prepared and stained with toluidine blue to further assess demyelination, as described (24).

Immunocytochemistry

Rehydrated paraffin wax sections were stained using an overnight, indirect immunoperoxidase/diaminobenzidine method, essentially as described (24). Endogenous peroxidase activity was blocked with hydrogen peroxide and sections were washed in tap water and equilibrated in 0.01 M PBS, pH 7.3, containing 0.6% BSA (PBS/BSA). Sections were then incubated overnight in a humidity chamber at 4°C, in the appropriate Ab diluted in PBS/ BSA, washed in PBS, and incubated for 1 h with the appropriate peroxi-
dase-conjugated secondary Ab diluted 1/100 in PBS/BSA. Sections were washed free of unbound conjugate and immersed for 5 min in a freshly made 0.05% solution of diaminobenzidine with 0.005% (v/v) hydrogen peroxide in PBS (0.1 M, pH 7.3). The sections were washed, counter-
stained with hematoxylin, dehydrated, and mounted in Xam (BDH/Merck, Coventry, U.K.). Sections immunostained with secondary conjugate alone were included with every experiment as controls.

Image analysis

Images were captured with a Hamamatsu digital camera and image analy-

Statistics

For comparisons between groups, Mann-Whitney U nonparametric tests were used; the exact two-tailed p-value corrected for ties is quoted through-
out. For correlation of demyelination with APP staining, nonparametric Spearman correlation was used.

Results

Characterization of C6-deficient (C6−) PVG/c and C6-sufficient (C6+) PVG/c rats

We established colonies of C6− and C6+ rats in house. C6 status was confirmed by standard complement hemolytic assay (CH50) and Western blotting using a polyclonal antiserum to rat C6. C6− rats had no detectable complement hemolytic activity, and C6 could not be detected in their serum by Western blotting (data not shown).

To reconstitute C6 activity in C6− rats, human C6 was purified to homogeneity from fresh frozen plasma by affinity chromatog-

Induction of EAE and ADEAE in PVG/c rats

The EAE and ADEAE models were already established in Lewis rats in our laboratories with a disease incidence of 100% using gpMBP in CFA as the encephalitogenic Ag. Various doses of en-

The Journal of Immunology 459

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clinical courses of EAE and ADEAE were then characterized in C6⁺/H11001 PVG/c rats (Fig. 1a). Rats with EAE reached a maximum average clinical score of 1.3 on day 13 and had almost completely recovered by day 19, whereas rats with ADEAE reached an average clinical score of 2.2 on day 14, 24 h postadministration of anti-MOG. In this group, three of five rats had to be sacrificed on day 14, as they had reached a clinical score of 3.

Disease was induced in a total of 64 PVG/c rats in the following experiments, 55 of which showed clinical disease, giving an overall incidence of 84%. Disease incidence in C6⁻ and C6⁺ rats was not significantly different, 82% (28/34) for C6⁻ compared with 90% (27/30) for C6⁺ rats, demonstrating that C6 deficiency had no effect on disease induction.

Comparison of disease profiles and pathology in C6⁻ and C6⁺ PVG/c rats

The EAE and ADEAE disease models were compared in C6⁻ and C6⁺ PVG/c rats. Fig. 1b shows the median clinical scores for each group on days 13 and 14 of disease induction. In the EAE model, C6⁻ and C6⁺ rats had comparable disease severity, indicating that C6 deficiency had no significant impact on the clinical course of EAE. By contrast, in the ADEAE model, there was a marked difference between C6⁺ and C6⁻ rats. The C6⁺ rats with ADEAE exhibited a significant increase in clinical score following administration of anti-MOG Ab on day 13 (day 13 vs day 14, \( p = 0.015 \), \( n = 6 \) per day), and on day 14, C6⁺ ADEAE rats had a significantly higher clinical score compared with the C6⁻ ADEAE rats (\( p = 0.009 \), \( n = 6 \) per group). The C6⁻ rats with ADEAE showed a clinical profile similar to that of EAE, indicating that the events triggered by administration of Ab were absent in C6 deficiency.

Histological analysis of demyelination

Spinal cord was taken from all of the rats in the above experiment. Analysis of myelin staining (LFB/CV method) in cervical spinal cord sections from rats with ADEAE demonstrated perivascular demyelination in spinal cord white matter of C6⁺ rats, but a complete absence of demyelination in C6⁻ rats (Fig. 2, compare a with b, and c with d). Image analysis permitted a quantitative assessment of demyelination in spinal cord (Fig. 2a). C6⁺ rats with ADEAE displayed high levels of demyelination (demyelination index 35%), whereas C6⁻ rats with ADEAE had no demyelination (demyelination index 1.7%). For both C6⁻ and C6⁺ rats with EAE, demyelination was absent.

Histological analysis of inflammation

The degree of inflammatory infiltrate was assessed in H&E-stained cervical spinal cord sections. Assessment of subpial and perivascular mononuclear cell infiltration by a blinded observer revealed no significant differences in the degree of infiltration between C6⁻ and C6⁺ rats with either EAE (not shown) or ADEAE (Fig. 2b). However, in C6⁻ rats, the infiltrate was localized to perivascular areas (“cuffing”), whereas in C6⁺ rats with ADEAE the infiltrate was more diffuse with mononuclear cells present throughout demyelinated areas as well as perivascular areas (Fig. 3, compare c with d). Semithin sections were stained with toluidine blue (Fig. 3, e and f) and further confirmed the localization of the infiltrate to perivascular areas in C6⁻ rats with ADEAE. Using this stain, it was possible to detect macrophages, containing myelin debris, in close apposition to axons in demyelinated areas of C6⁺ ADEAE rats (Fig. 3f, inset). Myelin containing macrophages were not found in C6⁻ ADEAE rats.

Immunocytochemical staining for T cells was performed in C6⁻ and C6⁺ rats with ADEAE using the W3/13 Ab, a marker of T cells and granulocytes. Mononuclear cells positive for this marker were counted, and the results were expressed as number of W3/13-positive T cells per square millimeter (Fig. 2c). No significant differences in the number of infiltrating T cells were observed.

Immunocytochemical staining for ED1, a monocyte/macrophage marker, was also performed to quantify the level of infil-

![FIGURE 1.](http://www.jimmunol.org/Downloadedfromhttp://www.jimmunol.org)
tration by this subpopulation. At least four spinal cord sections were analyzed per animal, and image analysis was performed, as described in Materials and Methods, to give an indication of the degree of staining (ED1 index). An average score was calculated for each animal, and then the group average was calculated (Fig. 2d). No significant differences were observed between C6− and C6+ rats with either EAE or ADEAE.

**Complement deposition in C6− and C6+ rats**

Deposition of C1 and MAC was assessed by immunocytochemical staining using polyclonal antisera to rat C1 and C9, respectively, as previously described (10). In C6− and C6+ rats with ADEAE, weak staining for C1 was detected throughout white matter, but in C6+ rats, enhanced staining was detected in perivascular and demyelinated areas, indicating higher levels of C1 deposition (Fig. 3, g and h). In C6+ rats with ADEAE, MAC deposition was detected as strong staining for C9 in areas of demyelination, whereas in C6− rats with ADEAE, there was no staining indicative of MAC deposition (Fig. 3, i and j).

**Axonal injury correlates with demyelination**

To investigate the relationship between myelin loss and axonal damage, demyelination and axonal APP staining were assessed in a blinded fashion by a second laboratory (Brain Research Institute, University of Vienna). Serial paraffin sections from C6− and C6+ rats with ADEAE were stained for APP and myelin (LFB) (Fig. 4, a, b, f, and g), and the degree of demyelination and axonal APP staining was quantified (Fig. 5, a and b). The results for demyelination were in agreement with our earlier results (compare Fig. 2a with 5a) and significantly different between the groups (p = 0.015, n = 6, Mann-Whitney U test). Axonal APP staining, indicating acute axonal injury, was significantly greater in C6+ ADEAE rats compared with C6− ADEAE rats (p = 0.015, n = 6 per group, Mann-Whitney U test) and correlated positively with demyelination (r = 0.86, p < 0.0005, n = 12, Spearman rank order correlation). Serial sections from C6+ ADEAE rats stained for phosphorylated neurofilament, and using Bielschowsky’s silver impregnation method for axons (Fig. 4, c and d), showed evidence of axonal fragmentation and swelling, in comparison with staining

**FIGURE 2.** Comparison of demyelination and inflammation in C6− and C6+ rats with EAE and ADEAE on day 14. Demyelination index (+SD) on day 14 of disease induction for each group of rats is shown in a, as determined by quantitative image analysis on LFB/CV-stained cervical spinal cord sections. Demyelination index measures the area of spinal cord white matter that does not stain for myelin. Significant demyelination was present only in C6− PVG/c rats with ADEAE; the C6− rats with ADEAE have minimal demyelination. The degree of perivascular and subpial infiltration for C6− and C6+ ADEAE rats on day 14 is shown as a dot plot in b, as assessed by a blinded observer on an arbitrary scale (bar represents median value; see Materials and Methods for scoring parameters). No significant differences in infiltration between C6− and C6+ rats with ADEAE were observed. c, Shows the numbers of W3/13-positive T cells/mm² in C6− and C6+ ADEAE rats on day 14; the bar represents median score (no significant differences between groups). d, Shows average ED1 index (+SE) determined by image analysis for each of the groups on day 14 (n = 6 per group, except C6− EAE, n = 3). Average score calculated for each animal from at least four spinal cord sections, and these scores were used to calculate the group average. ED1 is a marker of monocyte/macrophage infiltration; no significant differences were observed between C6− and C6+ rats with either EAE or ADEAE.
in C6⁻ ADEAE rats (Fig. 4, h and i), confirming that areas of demyelination contained significant axonal pathology. Finally, in the C6⁻ ADEAE rat, staining for C9 as an index of MAC deposition correlated exactly with the area showing axonal degeneration (Fig. 4e), whereas in the C6⁻ rat, C9 staining indicative of MAC deposition was absent (Fig. 4j). Weak, diffuse C9 staining was present in these animals, suggesting some leakage of serum C9 into the tissues.

Reconstitution of C6 in deficient rats restores disease and demyelination

It is reported that the C6⁻ PVG/c rats also have a partial deficiency of C2 (25). To confirm that the lack of demyelination in C6⁻ rats was due to a deficiency of C6 alone, ADEAE was induced in two groups (six in each) of C6⁻ rats, one of which was reconstituted with human C6 (8 mg/kg, i.v.) on day 13, while the other received PBS i.v. A third group comprising C6⁻ PVG/c rats was included as a control and also received PBS i.v. The clinical scores in all groups were similar on day 13. In the unreconstituted C6⁻ group, clinical score showed a small increase to day 14, whereas in animals reconstituted with C6, clinical score increased to a similar extent to that in C6⁻ rats (Fig. 6a). At day 14, the difference between the clinical scores in the C6⁻ control group and in the reconstituted group was significant (p = 0.026, n = 6). Staining for myelin in cervical spinal cord demonstrated that C6-reconstituted rats had significant perivascular demyelination, as observed...
in C6$^+$ rats, whereas no demyelination was present in the C6$^-$ rats that had received PBS alone (Fig. 5, b and c). No differences in perivascular or subpial mononuclear cell infiltration were observed between groups (data not shown). These data confirm that absence of C6 is solely responsible for reduced demyelination and disease in C6$^-$ animals.

**Discussion**

MS is an immune-mediated disease of the CNS; the central role of T cells in MS and the animal model EAE has been much emphasized. In EAE, transfer of encephalitogenic T cell populations is sufficient for disease induction in healthy animals (26). However, other immune effectors may be needed to achieve significant demyelination, the hallmark of MS (2). In particular, autoantibodies and complement play significant roles in tissue damage and demyelination in a subset of MS patients and demyelinating variants of EAE (17, 27, 28). In the rat EAE model, administration of anti-MOG Abs induced demyelination and exacerbated clinical disease, a complement-dependent process (10, 12). ADEAE thus provides a useful model for this large subgroup of MS patients. Although the role of complement in demyelination in humans and experimental animals is established, the causative factors are unidentified.

PVG/c rats were originally described as resistant to induction of EAE, with low incidence (29) or complete absence (30) of disease. Using our standard protocol for induction of EAE developed in Lewis rats, a disease incidence of 86% was achieved in PVG/c. These differences may relate to the higher amounts of *M. tuberculosis* used in the adjuvant in our protocol (31). EAE in PVG/c rats is a relatively mild disease, with most rats displaying only loss of tail tone with a low incidence of hind limb weakness or paralysis. Nevertheless, induction of ADEAE by administration of mAbs against MOG caused severe disease in this strain, similar to that seen in Lewis rats (10–12).

The C6$^+$ PVG/c rats have previously been used to demonstrate MAC dependence in animal models of hyperacute rejection (32), acute rejection (33), active Heymann nephritis (34), experimental mesangioproliferative glomerulonephritis (25), and endothelial cell apoptosis in experimental glomerulonephritis (35). In this study, we have used these rats to demonstrate that the MAC, while not essential for the induction of EAE, is responsible for demyelination and the associated axonal injury in ADEAE.

The incidence and severity of EAE in C6$^+$ and C6$^-$ PVG/c rats were similar, demonstrating that the MAC did not contribute significantly to pathology in this inflammatory model. Early studies using complement depletion have demonstrated a role of complement in EAE (9). It is therefore likely that the anaphylactic and opsonic fragments generated during the activation pathways contribute to inflammation in EAE. Further support for this hypothesis comes from analysis of C3 gene-targeted mice (C3$^{-/-}$), in which clinical disease, demyelination, and inflammation are reduced compared with wild-type controls in MOG peptide (p55–75)-induced EAE in C57BL/6 mice (13). Furthermore, factor B-deficient mice (B$^{-/-}$) showed similar reductions in disease severity, indicating a role for the alternative pathway in this EAE model. It should be noted, however, that the C3$^{-/-}$ observation has been contradicted recently. When C3$^{-/-}$ mice on a different background (BL/6 × 129/sv F1) were compared with wild-type littermates in

**FIGURE 5.** Axonal APP staining correlates with demyelination. Demyelination was quantified in representative sections for each rat and is shown as a dot plot of demyelinated area in square millimeters (a). The level of APP staining is expressed as APP-positive axons/mm$^2$ (b). Demyelination and axonal APP staining are significantly greater in C6$^+$ rats with ADEAE than in C6$^-$ rats (both tests, $p = 0.015, n = 6$, Mann-Whitney), and axonal APP staining correlates positively with demyelination (correlation coefficient = 0.86, $p < 0.0005$, Spearman rank order correlation).

**FIGURE 6.** Reconstitution of C6 exacerbates disease and induces demyelination. Clinical score on days 13 and 14 of disease induction is shown in a (six animals per group; bar represents median). Median clinical score for C6$^+$ rats reconstituted with human C6 (C6$^+$-HuC6) increases to a similar extent as for C6$^-$ rats after administration of anti-MOG Ab on day 13, and is significantly different from scores in C6$^-$ rats ($p = 0.026, n = 6$ per group) on day 14. Representative LFB/CV-stained cervical spinal cord sections are shown for a C6$^+$ rat with ADEAE (b) and a C6$^-$ rat with ADEAE that had been reconstituted with human C6 (c). Again, there is no demyelination in the control C6$^-$ rat, but in the C6-reconstituted rat, the pattern of demyelination is the same as in C6$^+$ rats (×50 magnification).
MOG-induced EAE, no significant differences in clinical disease, inflammation, or demyelination were detected (14).

Administration of anti-MOG Ab caused increased disease severity and induced demyelination in C6− rats, whereas in C6− PVG/c rats disease severity was not enhanced and demyelination was absent. Immunohistochemistry confirmed that MAC deposits were present only in C6− rats in areas of demyelination. In contrast, C1 deposition was present in both C6− and C6+ rats, but was much more abundant and focused in areas of demyelination in the latter group. The increased levels of C1 deposition seen in C6− rats suggest that initial damage and demyelination caused by the MAC expose C1 binding sites in myelin. This in turn will allow higher levels of C1 deposition and complement activation than are seen in C6− rats in which no myelin damage occurs. A similar observation was made in ADEAE rats treated with the complement inhibitor soluble human CR1 (12). Of note, soluble human CR1 also markedly reduced CNS inflammation in ADEAE, whereas C6− and C6+ rats in the present study had similar degrees of inflammatory infiltrate. Soluble CR1 acts early in the pathway and thus prevents generation of anaphylatoxins as well as formation of the MAC. It is therefore likely that the complement anaphylatoxins drive inflammation in these models.

Our findings demonstrate that the MAC (in which C6 is an essential component) is a critical effector in demyelination. These results contradict the suggestion that earlier components of the complement cascade mediate demyelination via opsonization of myelin with C3b and C4b and subsequent interaction of opsonins with complement receptors on macrophages or other phagocytic cells (36). It has been shown in vitro that opsonization of myelin by complement enhances phagocytosis by macrophages via CR3 (37), although an essential role for MAC in demyelination in vitro has also been suggested (38). In this study, it was not possible to detect demyelination in C6− rats with ADEAE and, in contrast to findings in C6− animals in which macrophages were phagocytosing myelin, no evidence of macrophage-mediated demyelination was seen despite unhindered early complement activation. We conclude that MAC-induced damage is essential for induction of demyelination; opsonins and phagocytes may then collaborate to remove the damaged myelin.

Immunocytochemical staining of axons for β amyloid precursor protein (APP) is a sensitive marker of early axonal damage, as APP accumulates at sites of axonal injury due to disruption of axonal transport and APP staining has been used as a marker of axonal injury in multiple sclerosis (39). APP staining correlated with demyelination in ADEAE and was negligible in C6− rats. This result is in agreement with studies in MS patients and MOG-induced chronic EAE in Lewis rats in which axonal APP staining was most pronounced in actively demyelinating plaques (40). Axonal damage as a consequence of demyelination or dysmyelination is a common finding (41), and axonal loss was described in the first observations of MS (42). The role of axonal damage in the pathology of MS has undergone a resurgence of interest. Although healthy axons may be able to compensate for myelin loss by increasing sodium channel density (43), the loss of axons is irreversible and may be responsible for persistent clinical disability (5). Whether axonal loss in the present study is directly mediated by MAC remains to be ascertained. Axons do not express MOG, and thus will not bind the complement-activating anti-MOG Ab. However, complement activation on myelin adjacent to the axon may cause bystander MAC deposition on axons; indeed, staining for C9 as an indicator of deposition of the MAC correlated very well with areas of axonal degeneration. Alternatively, axons, once exposed by demyelination, may directly activate complement, as was recently shown in vitro for human neurons (44). Human neurons are particularly susceptible to complement attack because they express only low levels of complement regulators on their membranes (44, 45). Rodent axons may be similarly compromised.

The data presented in this work provide compelling evidence for a role of the MAC in demyelination and raise the possibility of using specific inhibitors of the membrane attack pathway as therapy in the human demyelinating disease, MS. Some inhibitors based on blockade of the C5 cleavage step in the complement cascade are already in clinical trial for a number of diseases, including cardiopulmonary bypass and rheumatoid arthritis (46). Such inhibitors may prove particularly useful in subtypes of MS in which complement plays a significant role in pathology (17).

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